

AMENDMENT

In the specification

Please replace the paragraph beginning at page 20, line 4, with the following rewritten paragraph:

**--VIII. Growth Hormone Variants and Methods of Use**

D1 The cloned gene for hGH has been expressed in a secreted form in Escherichia coli (Chang, C. N., *et al.*, [1987] Gene 55, 189) and its DNA and amino acid sequence has been reported (Goeddel, *et al.* [1979] Nature 281, 544; Gray *et al.*, [1985] Gene 39, 247). The present invention describes novel hGH variants produced using the phagemid selection methods. Human growth hormone variants containing substitutions at positions 10, 14, 18, 21, 167, 171, 172, 174, 175, 176, 178 and 179 have been described. Those having higher binding affinities are described in Tables VII, XIII and XIV. The amino acid nomenclature for describing the variants is shown below. Growth hormone variants may be administered and formulated in the same manner as regular growth hormone. The growth hormone variants of the present invention may be expressed in any recombinant system which is capable of expressing native or met hGH.--

Please replace the paragraph beginning at page 7, line 11, with the following rewritten paragraph:

D2 --FIGURE 5. Amino acid substitutions at positions 172, 174, 176 and 178 of hGH (The notation, e.g. KSYR, denotes hGH mutant 172K/174S/176Y/178R.) found after sequencing a number of clones from rounds 1 and 3 of the selection process for the pathways indicated (hGH elution; Glycine elution; or Glycine elution after pre-adsorption). Non-functional sequences (i.e. vector background, or other prematurely terminated and/or frame-shifted mutants) are shown as "NF". Functional sequences which contained a non-silent, spurious mutation (i.e. outside the set of target residues) are marked with a "+". Protein sequences which appeared more than once among all the sequenced clones, but with different DNA sequences, are marked with a "#". Protein sequences which appeared more than once among the sequenced clones and with the same DNA sequence are marked with a "\*". Note that after three rounds of selection, 2 different contaminating sequences were found; these clones did not correspond to cassette mutants, but to previously constructed hormone phage. The pS0643 contaminant corresponds to wild-type hGH-phage (hGH "KEFR"(SEQ ID NO:44)). The pH0457 contaminant, which dominates the third-round glycine-selected pool of phage, corresponds to a previously identified mutant

Document # 131840

D2 of hGH, "KSYR." The amplification of these contaminants emphasizes the ability of the hormonephage selection process to select for rarely occurring mutants. The convergence of sequences is also striking in all three pathways: R or K occurs most often at positions 172 and 178; Y or F occurs most often at position 176; and S, T, A, and other residues occur at position 174.--

Please replace the paragraph beginning at page 7, line 31, with the following rewritten paragraph:

D3 --FIGURE 7. Sequences from phage selected on hPRLbp-beads in the absence of zinc. The notation is as described in Figure 5. In contrast to the sequences of Figure. 6, these sequences appear more hydrophilic. After 4 rounds of selection using hGH elution, two clones (ANHQ (SEQ ID NO:45), and TLDT/171V (SEQ ID NO:108)) dominate the pool.--

Please replace Table VI, beginning at page 34, line 1, with the following rewritten Table VI:

--Table VI.

Non-selected (pH0529E) clones with an open reading frame.

The notation, e.g. TWGS, denotes the hGH mutant 172T/174W/176G/178S. Amber (TAG) codons, translated as Glu in XL1-Blue cells are shown as  $\epsilon$ .

D4

K $\epsilon$ NT (SEQ ID NO: 46)	KTEQ (SEQ ID NO: 59)	CVLQ (SEQ ID NO:72 )
TWGS (SEQ ID NO: 47)	NNCR (SEQ ID NO: 60)	EASL (SEQ ID NO: 73)
P $\epsilon$ ER (SEQ ID NO: 48)	FPCL (SEQ ID NO: 61)	SSKE (SEQ ID NO: 74)
LPPS (SEQ ID NO: 49)	NSDF (SEQ ID NO: 62)	ALLL (SEQ ID NO: 75)
SLDP (SEQ ID NO: 50)	HRPS (SEQ ID NO: 63)	PSHP (SEQ ID NO: 76)
QQSN (SEQ ID NO: 51)	LSL $\epsilon$ (SEQ ID NO: 64)	SYAP (SEQ ID NO: 77)
GSKT (SEQ ID NO: 52)	NGSK (SEQ ID NO: 65)	ASNG (SEQ ID NO: 78)
TPVT (SEQ ID NO: 53)	LTTE (SEQ ID NO: 66)	EANN (SEQ ID NO: 79)
RSRA (SEQ ID NO: 54)	PSGG (SEQ ID NO: 67)	KNAK (SEQ ID NO: 80)
LCGL (SEQ ID NO: 55)	LWFP (SEQ ID NO: 68)	SRGK (SEQ ID NO: 81)
TGRL (SEQ ID NO: 56)	PAGS (SEQ ID NO: 69)	GLDG (SEQ ID NO: 82)
AKAS (SEQ ID NO: 57)	GRAK (SEQ ID NO: 70)	NDPI (SEQ ID NO: 83)
GNDD (SEQ ID NO: 58)	GTNG (SEQ ID NO: 71)	

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Please replace the paragraph beginning at page 36, line 4 with the following rewritten paragraph:

D5 --The results for a number of hGH mutants, selected by different pathways (Fig. 6) are shown in Table VII. Many of these mutants have a tighter binding affinity for hGHbp than wild-type hGH. The

D5 most improved mutant, KSYR (SEQ ID NO:84), has a binding affinity 5.6 times greater than that of wild-type hGH. The weakest selected mutant, among those assayed was only about 10-fold lower in binding affinity than hGH.--

Please replace the paragraph beginning at page 36, line 48 with the following rewritten paragraph:

**--Additive and non-additive effects on binding**

D6 At some residues, substitution of a particular amino acid has essentially the same effect independent of surrounding residues. For example, substitution of F176Y in the background of 172R/174S reduces binding affinity by 2.0-fold (RSFR (SEQ ID NO:85) vs. RSYR (SEQ ID NO:88)). Similarly, in the background of 172K/174A the binding affinity of the F176Y mutant (KAYR (SEQ ID NO:89)) is 2.9-fold weaker than the corresponding 176F mutant (KAFR; Cunningham and Wells, 1989).-

Please replace the paragraph beginning at page 37, line 1 with the following rewritten paragraph:

D7 --On the other hand, the binding constants determined for several selected mutants of hGH demonstrate non-additive effects of some amino acid substitutions at residues 172, 174, 176, and 178. For example, in the background of 172K/176Y, the substitution E174S results in a mutant (KSYR (SEQ ID NO:84)) which binds hGHbp 3.7-fold tighter than the corresponding mutant containing E174A (KAYR (SEQ ID NO:89)). However, in the background of 172R/176Y, the effects of these E174 substitutions are reversed. Here, the E174A mutant (RAYR (SEQ ID NO:86)) binds 1.5-fold tighter than the E174S mutant (RSYR (SEQ ID NO:88)).--

Please replace Table VII, beginning at page 36, line 10, with the following rewritten Table VII:

**--Table VII.  
Competitive binding to hGHbp**

The selected pool in which each mutant was found is indicated as 1G (first glycine selection), 3G (third glycine selection), 3H (third hGH selection), 3\* (third selection, not binding to hPRLbp, but binding to hGHbp). The number of times each mutant occurred among all sequenced clones is shown ().

D8

Mutant	Kd (nM)	Kd(mut)/Kd(hGH)	Pool
KSYR (6) (SEQ ID NO:84)	0.06 + 0.01	0.18	1G,3G
RSFR (SEQ ID NO:85)	0.10 + 0.05	0.30	3G

Document # 131840

Serial No. 09/717,641

RAYR (SEQ ID NO:86)	0.13 + 0.04	0.37	3*
KTYK (2) (SEQ ID NO:87)	0.16 + 0.04	0.47	H,3G
RSYR (3) (SEQ ID NO:88)	0.20 + 0.07	0.58	1G,3H,3G
KAYR (3) (SEQ ID NO:89)	0.22 + 0.03	0.66	3G
RFFR (2) (SEQ ID NO:90)	0.26 + 0.05	0.76	3H
KQYR (SEQ ID NO:91)	0.33 + 0.03	1.0	3G
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KEFR= wt (9)	0.34 + 0.05	1.0	3H,3G,3*
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RTYH (SEQ ID NO:92)	0.68 + 0.17	2.0	3H
QRYR (SEQ ID NO:93)	0.83 + 0.14	2.5	3*
KKYK (SEQ ID NO:94)	1.1 + 0.4	3.2	3*
RSFS (2) (SEQ ID NO:95)	1.1 + 0.2	3.3	3G,*
KSNR (SEQ ID NO:96)	3.1 + 0.4	9.2	3*
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Please replace Table B1, beginning at page 60, line 1, with the following rewritten Table B1:

**--Table B1**

**Sequences of eluted phage after 2 rounds of selective enrichment.**

All protein sequences should be of the form AA\*\*TRQ, where \* represents a randomised codon. In the table below, the randomised codons and amino acids are underlined and in bold.

After round 2:

<u>Sequence</u>	<u>No. of</u> <u>occurrences</u>
*   *	
A   A <u>H</u> <u>Y</u> T   R   Q (SEQ ID NO:97)	
... GCT GCT <u>CAC TAC</u> ACC CGG CAA ... (SEQ ID NO:32) 2	
A   A <u>H</u> <u>M</u> T   R   Q (SEQ ID NO:98)	
... GCT GCT <u>CAC ATG</u> ACC CGG CAA ... (SEQ ID NO:33) 1	
A   A <u>L</u> <u>H</u> T   R   Q (SEQ ID NO:99)	
... GCT GCT <u>CTC CAC</u> ACC CGG CAA ... (SEQ ID NO:34) 1	
A   A <u>L</u> <u>H</u> T   R   Q (SEQ ID NO:99)	
... GCT GCT <u>CTG CAC</u> ACC CGG CAA ... (SEQ ID NO:35) 1	
A   A <u>H</u> <u>T</u> R   Q   (SEQ ID NO:100)	
... GCT GCT <u>CAC ACC</u> CGG CAA ... (SEQ ID NO:36) 1   #	
A   A <u>?</u> <u>H</u> T   R   Q (SEQ ID NO:101)	
... GCT GCT <u>??? CAC</u> ACC CGG CAA (SEQ ID NO:37) 1   ##	
... wild-type pDM0454	3

# - spurious deletion of 1 codon within the cassette

## - ambiguous sequence--

Please replace Table B2 beginning at page 61, line 1, with the following rewritten Table B2:

--Table B2

Sequences of eluted phage after 3 rounds of selective enrichment.

All protein sequences should be of the form AA\*\*TRQ, where \* represents a randomised codon. In the table below, the randomised codons and amino acids are underlined and in bold.

After round 3:

<u>Sequence</u>	<u>No. of occurrences</u>
<div style="text-align: center;">*      *</div> A   A <u><b>H</b></u> <u><b>Y</b></u> T   R   Q (SEQ ID NO:97) ... GCT GCT <u><b>CAC TAT</b></u> ACC CGT CAG ... (SEQ ID NO:38) 2      #	
A   A <u><b>L</b></u> <u><b>H</b></u> T   R   Q (SEQ ID NO:99) ... GCT GCT <u><b>CTC CAC</b></u> ACC CGG CAA ... (SEQ ID NO:34) 2	
A   A <u><b>Q</b></u> <u><b>H</b></u> T   R   Q (SEQ ID NO:102) ... GCT GCT <u><b>CAG CAC</b></u> ACC CGG CAA ... (SEQ ID NO:39) 1	
A   A <u><b>T</b></u> <u><b>H</b></u> T   R   Q (SEQ ID NO:103) ... GCT GCT <u><b>ACG CAC</b></u> ACC CGG CAA ... (SEQ ID NO:40) 1	
A   A <u><b>H</b></u> <u><b>S</b></u> R   Q (SEQ ID NO:104) ... GCT GCT <u><b>CAC TCC</b></u> CGG CAA ... (SEQ ID NO:41) 1	
A   A <u><b>H</b></u> <u><b>H</b></u> T   R   Q (SEQ ID NO:105) ... GCT GCT <u><b>CAT CAT</b></u> ACC CGG CAA (SEQ ID NO:42) 1      ##	
A   A <u><b>H</b></u> <u><b>F</b></u> R   Q (SEQ ID NO:106) ... GCT GCT <u><b>CAC TTC</b></u> CGG CAA ... (SEQ ID NO:43) 1	
A   A <u><b>H</b></u> <u><b>T</b></u> R   Q (SEQ ID NO:100) ... GCT GCT <u><b>CAC ACC</b></u> CGG CAA ... (SEQ ID NO:36) 1	

# - contaminating sequence from pDM0411

## - contains the "illegal" codon CAT - T should not appear in the 3rd position of a codon.--